

What is claimed is:

1. An electrochemical sensor for measuring a first analyte in a sample, said sensor comprising:
  - at least a first working electrode having a first electrode sensing portion; and
  - 5 a first electrode material disposed on said first electrode sensing portion, said first electrode material being a mixture made by combining at least one enzyme wherein said at least one enzyme is capable of reacting with said first analyte, a redox mediator capable of reacting with a product of an enzymatic reaction, and a peroxidase capable of catalyzing the reaction involving said redox mediator and said product of said enzymatic reaction wherein said redox mediator is oxidized.
- 10 2. The sensor of Claim 1 wherein said first electrode material for measuring said analyte is configured for measuring one of creatine, glucose and cholesterol.
- 15 3. The sensor of Claim 1 further comprising a reference electrode having an active reference portion and a reference electrode material disposed on said active reference portion wherein said reference electrode material is selected from the group consisting of silver chloride when said active reference portion is silver and a mixture made by combining at least said redox mediator and at least one binder when said active reference portion is selected from the group consisting of gold, gold/tin oxide, palladium, platinum and carbon composition.
- 20 4. The sensor of Claim 2 further comprising a second working electrode having a second electrode portion and a second electrode material disposed on said second electrode portion when said first electrode material is configured for measuring creatine, said second electrode material being a mixture made by combining said at least one enzyme, a second electrode material enzyme
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capable of reacting with a second analyte in said sample forming said first analyte, said redox mediator, and said peroxidase.

5. The sensor of Claim 1 wherein said redox mediator is at least one of  $\text{Fe}(\text{CN})_6^{3-}$ ,  $\text{Fe}(\text{CN})_6^{4-}$ ,  $\text{Fe}(1,10\text{-phenanthroline})_3^{2+}$ ,  $\text{Fe}(2,2'\text{-bipyridine})_3^{2+}$ ,  $\text{Co}(\text{NH}_3)_6^{2+}$ ,  $\text{Co}(1,10\text{-phenanthroline})_3^{2+}$ ,  $\text{Co}(2,2'\text{-bipyridine})_3^{2+}$ ,  $\text{Os}(2,2'\text{-bipyridine})_2\text{Cl}^+$ ,  $\text{Os}(1,10\text{-phenanthroline})_2\text{Cl}^+$ ,  $\text{Ru}(2,2'\text{-bipyridine})_2^{2+}$ ,  $\text{Rh}(2,2'\text{-bipyridine})_2^{2+}$ , cobalt phthalocyanine, ferrocenes, methylene blue, methylene green, 7,7,8,8-tetracyanoquinodimethane, tetrathiafulvalene, toluidine blue, meldonium blue, N-methylphenazine methosulfate, phenyldiamines, 3,3',5,5'-tetramethylbenzidine, pyrogallol, and benzoquinone.
10. The sensor of Claim 5 wherein said redox mediator is potassium ferrocyanide.
7. The sensor of Claim 1 wherein said peroxidase is at least one of soybean peroxidase and horseradish root peroxidase.
15. The sensor of Claim 1 wherein said at least one enzyme is one of creatine amidinohydrolase, glucose oxidase and cholesterol oxidase.
9. The sensor of Claim 8 wherein said first electrode material further includes a second enzyme when said at least one enzyme is one of creatine amidinohydrolase and cholesterol oxidase.
10. The sensor of Claim 9 wherein said second enzyme is sarcosine oxidase when said at least one enzyme is creatine amidinohydrolase.
20. The sensor of Claim 9 wherein said second enzyme is cholesterol esterase when said at least one enzyme is cholesterol oxidase.

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12. The sensor of Claim 4 wherein said at least one enzyme is creatine amidinohydrolase, said second electrode material enzyme is creatinine amidohydrolase and said peroxidase is at least one of soybean peroxidase and horseradish root peroxidase.
- 5 13. The sensor of Claim 12 wherein said second electrode material further includes sarcosine oxidase.
14. The sensor of Claim 1 wherein said first electrode material further includes at least one binder and a surfactant.
- 10 15. The sensor of Claim 14 wherein said at least one binder is at least one of methylcellulose and polyethylene oxide.
- 15 16. The sensor of Claim 1 wherein said first electrode material is made from a mixture having starting components in water comprising about 2wt% of potassium ferrocyanide, about 1wt% of methyl cellulose, about .02wt% of said t-octylphenoxyethoxyethanol, about 0.5wt% of glucose oxidase, and about 0.5wt% of soybean peroxidase.
- 20 17. The sensor of Claim 1 wherein said first electrode material is made from a mixture having starting components in water comprising about 5wt% of potassium ferrocyanide, about 1wt% of methyl cellulose, about .02wt% of t-octylphenoxyethoxyethanol, about 2wt% of cholesterol oxidase, about 1wt% of cholesterol esterase, and about 0.5wt% of soybean peroxidase.
18. The sensor of Claim 4 wherein said first electrode material is made from a mixture having starting components in water comprising about 2wt% of potassium ferrocyanide, about 1wt% of methyl cellulose, about .02wt% of t-

octylphenoxypolyethoxyethanol, about 2wt% of creatine amidinohydrolase, about 0.5wt% of sarcosine oxidase, and about 0.5wt% of soybean peroxidase, and wherein said second working electrode material is made from a mixture having starting components in water comprising about 2wt% of said potassium ferrocyanide, about 1wt% of said methyl cellulose, about .02wt% of said t-octylphenoxypolyethoxyethanol, about 2wt% of said creatine amidinohydrolase, about 0.4wt% of creatinine amidohydrolase, about 0.5wt% of said sarcosine oxidase, and about 0.5wt% of said soybean peroxidase.

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19. The sensor of Claim 1 wherein said first electrode material further includes an antioxidant.
  20. A disposable electrode strip for measuring an analyte in a fluid sample, said strip comprising:  
a laminated strip having a first strip end, a second strip end and a vent opening spaced from said first strip end, said laminated strip comprising a base layer with at least two electrodes delineated thereon, a reagent holding layer carried on said base layer, said reagent holding layer having at least two cutouts, a channel forming layer carried on said reagent holding layer, and a cover;
  - an enclosed channel between said first strip end and said vent opening, said enclosed channel containing said at least two cutouts;
  - a first reagent disposed in a first cutout of said at least two cutouts forming a reference electrode, said first reagent comprising a reference electrode material selected from the group consisting of silver chloride when said reference electrode is silver and a mixture made by combining at least a redox mediator and at least one binder when said reference electrode is

- selected from the group consisting of gold, gold/tin oxide, palladium, platinum and carbon composition;
- a second reagent disposed in a second cutout of said at least two cutouts forming a first working electrode, said second reagent comprising a redox mediator, at least one binder, at least one enzyme that is a substrate of said analyte and a peroxidase capable of catalyzing a reaction involving said redox mediator wherein said redox mediator is oxidized; and
- 5 conductive contacts at said second strip end and insulated from said enclosed channel.
- conducting contacts at said second strip end and insulated from said enclosed channel.
- 10 21. The electrode strip of Claim 20 further comprising a third cutout and a third reagent disposed in said third cutout forming a second working electrode wherein said third reagent comprises said redox mediator and said at least one binder.
- 15 22. The electrode strip of Claim 21 wherein said third reagent further includes said at least one enzyme, a substrate of said at least one enzyme and a peroxidase.
23. The electrode strip of Claim 20 wherein said peroxidase is at least one of soybean peroxidase and horseradish root peroxidase.
24. The electrode strip of Claim 20 wherein said at least one enzyme is one of creatine amidinohydrolase, glucose oxidase and cholesterol oxidase.
- 20 25. The electrode strip of Claim 24 wherein said second reagent further includes a second enzyme when said at least one enzyme is one of creatine amidinohydrolase and cholesterol oxidase.

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- 26. The electrode strip of Claim 25 wherein said second enzyme is sarcosine oxidase when said at least one enzyme is creatine amidinohydrolase.
  - 27. The electrode strip of Claim 25 wherein said second enzyme is cholesterol esterase when said at least one enzyme is cholesterol oxidase.
  - 5 28. The electrode strip of Claim 20 wherein said redox mediator is an inorganic or organic redox species.
  - 29. The electrode strip of Claim 28 wherein said redox species is at least one of  $\text{Fe}(\text{CN})_6^{3-}$ ,  $\text{Fe}(\text{CN})_6^{4-}$ ,  $\text{Fe}(1,10\text{-phenanthroline})_3^{2+}$ ,  $\text{Fe}(2,2'\text{-bipyridine})_3^{2+}$ ,  $\text{Co}(\text{NH}_3)_6^{2+}$ ,  $\text{Co}(1,10\text{-phenanthroline})_3^{2+}$ ,  $\text{Co}(2,2'\text{-bipyridine})_3^{2+}$ ,  $\text{Os}(2,2'\text{-bipyridine})_2\text{Cl}^+$ ,  $\text{Os}(1,10\text{-phenanthroline})_2\text{Cl}^+$ ,  $\text{Ru}(2,2'\text{-bipyridine})_2^{2+}$ ,  $\text{Rh}(2,2'\text{-bipyridine})_2^{2+}$ , cobalt phthalocyanine, ferrocenes, methylene blue, methylene green, 7,7,8,8-tetracyanoquinodimethane, tetrathiafulvalene, toluidine blue, meldonia blue, N-methylphenazine methosulfate, phenyldiamines, 3,3',5,5'-tetramethylbenzidine, pyrogallol, and benzoquinone.
  - 15 30. The electrode strip of Claim 29 wherein said redox mediator is potassium ferrocyanide.
  - 31. The electrode strip of Claim 20 wherein said enclosed channel is hydrophilic.
  - 32. The electrode strip of Claim 20 wherein said enclosed channel has a volume of about 1.5 microliters.
  - 20 33. The electrode strip of Claim 20 wherein said cover has a hydrophilic coating on at least one side.

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34. The electrode strip of Claim 21 wherein said first reagent, said second reagent and said third reagent are made from a mixture having starting components comprising about 1wt% to about 6.5wt% of said redox mediator, about 1wt% of said binder, and about .02wt% of said surfactant in water.
- 5 35. The electrode strip of Claim 34 wherein said first reagent, said second reagent and said third reagent further includes about 0.05wt% to about 0.1wt% of an antioxidant.
- 10 36. The electrode strip of Claim 34 wherein said second reagent is made from a mixture having starting components in water comprising about 2wt% of potassium ferrocyanide, about 1wt% of methyl cellulose, about .02wt% of said t-octylphenoxyethoxyethanol, about 0.5wt% of glucose oxidase, and about 0.5wt% of soybean peroxidase.
- 15 37. The electrode strip of Claim 34 wherein said second reagent is made from a mixture having starting components in water comprising about 5wt% of potassium ferrocyanide, about 1wt% of methyl cellulose, about .02wt% of t-octylphenoxyethoxyethanol, about 2wt% of cholesterol oxidase, about 1wt% of cholesterol esterase, and about 0.5wt% of soybean peroxidase.
- 20 38. The electrode strip of Claim 34 wherein said second reagent is made from a mixture having starting components in water comprising about 2wt% of potassium ferrocyanide, about 1wt% of methyl cellulose, about .02wt% of t-octylphenoxyethoxyethanol, about 2wt% of creatine amidinohydrolase, about 0.5wt% of sarcosine oxidase, and about 0.5wt% of soybean peroxidase, and wherein said third reagent is made from a mixture having starting components in water comprising about 2wt% of said potassium ferrocyanide, about 1wt% of said methyl cellulose, about .02wt% of said t-
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octylphenoxypolyethoxyethanol, about 2wt% of said creatine amidinohydrolase, about 0.4wt% of creatinine amidohydrolase, about 0.5wt% of said sarcosine oxidase, and about 0.5wt% of said soybean peroxidase.

- 5        39. The electrode strip of Claim 38 wherein said first reagent and said second  
reagent further includes about 0.05wt% of an antioxidant.

10      40. The electrode strip of Claim 20 wherein said first reagent and said second  
reagent are made from a mixture having starting components in water  
comprising about 2wt% of potassium ferrocyanide, about 1wt% of methyl  
cellulose, about .02wt% of said t-octylphenoxypolyethoxyethanol, about 0.5wt%  
of glucose oxidase, and about 0.5wt% of soybean peroxidase.

15      41. The electrode strip of Claim 40 wherein said first reagent and said second  
reagent further includes about 0.1wt% of an antioxidant.

20      42. The electrode strip of Claim 20 wherein said first reagent and said second  
reagent are made from a mixture having starting components in water  
comprising about 5wt% of potassium ferrocyanide, about 1wt% of methyl  
cellulose, about .02wt% of t-octylphenoxypolyethoxyethanol, about 2wt% of  
cholesterol oxidase, about 1wt% of cholesterol esterase, and about 0.5wt% of  
soybean peroxidase.

25      43. The electrode strip of Claim 42 wherein said first reagent and said second  
reagent further includes about 0.1wt% of an antioxidant.

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44. The electrode strip of Claim 20 wherein said channel forming layer has a thickness sufficient to optimize the flow of said fluid sample along said open path.
45. The electrode strip of Claim 21 wherein the surface area of said first working electrode is substantially the same size as the surface area of said second working electrode.
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46. A disposable electrode strip for measuring an analyte in a fluid sample, said strip comprising:
- 10 a laminated strip having a first strip end, a second strip end and a vent opening spaced from said first strip end, said laminated strip comprising a base layer with at least two electrodes delineated thereon, a channel forming layer carried on said base layer, and a cover;
- 15 an enclosed channel between said first strip end and said vent opening, said enclosed channel sized to hold a volume of said fluid sample less than one microliter;
- 20 a reagent matrix disposed on said base layer in said enclosed channel, said reagent matrix containing at least one enzyme that is a substrate of said analyte, a redox mediator and a peroxidase capable of catalyzing a reaction involving said redox mediator wherein said redox mediator is oxidized; and
- conductive contacts at said second strip end and insulated from said enclosed channel.
47. The electrode strip of Claim 46 wherein said peroxidase is at least one of soybean peroxidase and horseradish root peroxidase.

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48. The electrode strip of Claim 46 wherein said at least one enzyme is one of glucose oxidase and cholesterol oxidase.
49. The electrode strip of Claim 48 wherein said reagent matrix further includes a second enzyme when said at least one enzyme is cholesterol oxidase.
- 5 50. The electrode strip of Claim 49 wherein said second enzyme is cholesterol esterase when said at least one enzyme is cholesterol oxidase.
51. The electrode strip of Claim 46 wherein said redox mediator is an inorganic or organic redox species.
52. The electrode strip of Claim 51 wherein said redox species is at least one of 10  $\text{Fe}(\text{CN})_6^{3-}$ ,  $\text{Fe}(\text{CN})_6^{4-}$ ,  $\text{Fe}(1,10\text{-phenanthroline})_3^{2+}$ ,  $\text{Fe}(2,2'\text{-bipyridine})_3^{2+}$ ,  $\text{Co}(\text{NH}_3)_6^{2+}$ ,  $\text{Co}(1,10\text{-phenanthroline})_3^{2+}$ ,  $\text{Co}(2,2'\text{-bipyridine})_3^{2+}$ ,  $\text{Os}(2,2'\text{-bipyridine})_2\text{Cl}^+$ ,  $\text{Os}(1,10\text{-phenanthroline})_2\text{Cl}^+$ ,  $\text{Ru}(2,2'\text{-bipyridine})_2^{2+}$ ,  $\text{Rh}(2,2'\text{-bipyridine})_2^{2+}$ , cobalt phthalocyanine, ferrocenes, methylene blue, methylene green, 7,7,8,8-tetracyanoquinodimethane, tetrathiafulvalene, toluidine blue, 15 meldola blue, N-methylphenazine methosulfate, phenyldiamines, 3,3',5,5'-tetramethylbenzidine, pyrogallol, and benzoquinone.
53. The electrode strip of Claim 52 wherein said redox mediator is potassium ferrocyanide.
54. The electrode strip of Claim 46 wherein said reagent matrix is made from a 20 mixture having starting components comprising about 1wt% to about 6.5wt% of said redox mediator, about 1wt% of said binder, and about .02wt% of said surfactant in water.

55. The electrode strip of Claim 54 wherein said reagent matrix further includes about 0.05wt% to about 0.1wt% of an antioxidant.
56. The electrode strip of Claim 54 wherein said reagent matrix is made from a mixture having starting components in water comprising about 2wt% of potassium ferrocyanide, about 1wt% of methyl cellulose, about .02wt% of said t-octylphenoxyethoxyethanol, about 0.5wt% of glucose oxidase, and about 0.5wt% of soybean peroxidase.
57. The electrode strip of Claim 56 wherein said reagent matrix further includes about 0.1wt% of an antioxidant.
- 10 58. The electrode strip of Claim 54 wherein said reagent matrix is made from a mixture having starting components in water comprising about 5wt% of potassium ferrocyanide, about 1wt% of methyl cellulose, about .02wt% of t-octylphenoxyethoxyethanol, about 2wt% of cholesterol oxidase, about 1wt% of cholesterol esterase, and about 0.5wt% of soybean peroxidase.
- 15 59. The electrode strip of Claim 58 wherein said reagent matrix further includes about 0.1wt% of an antioxidant.
60. The electrode strip of Claim 46 wherein said channel forming layer has a thickness sufficient to optimize the flow of said fluid sample along said open path.
- 20 61. A method of using an electrode strip for determining the concentration of creatinine, said electrode strip having a first working electrode, a second working electrode and a reference electrode wherein said first working electrode contains a first enzyme capable of catalyzing a reaction involving a

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- substrate for creatine and said second working electrode contains a second enzyme capable of catalyzing a reaction involving a substrate for creatinine, said first working electrode, said second working electrode and said reference electrode being disposed in a fluid sample channel for measuring a fluid sample, said method comprising:
- 5 disposing said fluid sample into said channel of said electrode strip;
- applying a potential between said reference electrode and said first working electrode;
- measuring a first current generated between said first working electrode and said reference electrode and correlating said first current to a concentration of said creatine in said fluid sample;
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- applying a potential between said reference electrode and said second working electrode;
- measuring a second current generated between said second working electrode and said reference electrode;
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- subtracting said first current from said second current and obtaining a current difference whereby said current difference is representative of the concentration of creatinine in said fluid sample.
- 20 **62.** The method of Claim 61 wherein said method further includes triggering said current measuring step when said fluid sample contacts said first working electrode, said second working electrode and said reference electrode creating said first current and said second current.
- 63.** The method of Claim 61 wherein said method further includes reading a current value for each of said first current and said second current at about a time
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where said current values for each of said first current and said second current reach a steady-state.

64. The method of Claim 63 wherein said reading is taken at about 20 seconds after said current measuring step is triggered.
- 5     **65.** A method of making a creatinine sensor wherein said sensor has a first working electrode, a second working electrode and a reference electrode, wherein said first working electrode contains a first reagent material, said second working electrode contains a second reagent material and said reference electrode contains a reference reagent material, said first working electrode, said second working electrode and said reference electrode being disposed in a fluid sample channel for measuring a fluid sample, said method comprising:
- 10           obtaining a base strip of an insulating material having a layer of conductive material disposed thereon, said base strip having a first end and a second end;
- 15           scribing in said conductive material a plurality of lines in a predetermined pattern forming three conductive paths between said first end and said second end;
- 20           disposing a first middle layer of insulating material over said base strip, said first middle layer having three cutouts wherein each cutout exposes an electrode portion of each of said three conductive paths wherein said three cutouts are spaced from said first end of said base strip, and wherein said first middle layer is sized to expose a contact portion of each of said three conductive paths for a distance from said second end of said base strip;
- 25           disposing said first reagent material on one of said three cutouts, said second reagent material on a second of said three cutouts and said reference reagent material on a third of said three cutouts, wherein said reference

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reagent material is made from mixing in water at least a redox mediator,  
said first reagent material is made from mixing in water a first and second  
enzyme, a redox mediator capable of reacting with a product of an  
enzymatic reaction involving said first and second enzyme, a peroxidase  
capable of catalyzing a reaction involving said redox mediator and said  
product wherein said redox mediator is oxidized, and said second reagent  
material is made from by mixing in water said first and second enzyme, a  
third enzyme, said redox mediator capable of reacting with a product of an  
enzymatic reaction involving said first and second enzyme and said third  
enzyme, said peroxidase capable of catalyzing a reaction involving said  
redox mediator and said product wherein said redox mediator is oxidized;  
drying said first reagent material, said second reagent material and said  
reference reagent material;  
overlaying a second middle layer of insulating material over and coextensive  
with said first middle layer, said second middle layer having an elongated  
cutout portion where said elongated cutout portion exposes said three  
cutouts of said first middle layer; and  
disposing a top layer of insulating material over and coextensive with said  
second middle layer, said top layer having a vent opening wherein said  
vent opening exposes a portion of said elongated cutout portion furthest  
from said first end of said base strip.

66. The method of Claim 65 further comprising drying said first reagent material,  
said second reagent material and said third reagent material at a  
predetermined temperature and for a predetermined length of time sufficient to  
allow said first reagent material, said second reagent material and said

reference reagent material to solidify and adhere to each of said electrode portions of said three conductive paths.

- 5        67. A method of making multiple, disposable creatinine sensors wherein each sensor has a first working electrode, a second working electrode and a reference electrode, wherein said first working electrode contains a first reagent material, said second working electrode contains a second reagent material and said reference electrode contains a reference reagent material, said first working electrode, said second working electrode and said reference electrode being disposed in a fluid sample channel for measuring a fluid sample, said 10 method comprising:

obtaining a base strip of an insulating material having a layer of conductive material disposed thereon, said base strip having a first edge and a second edge;

scribing in said conductive material a plurality of lines in a repetitive pattern wherein said plurality of lines contain a repetitive pattern forming three conductive paths in each of said repetitive pattern;

disposing a first middle layer of insulating material over said base strip, said first middle layer having a repetitive pattern of three cutouts wherein each cutout of each of said repetitive pattern exposes an electrode portion of each of said three conductive paths of each repetitive pattern wherein said repetitive pattern of said three cutouts are spaced from said first edge of said base strip, and wherein said first middle layer is sized to expose a contact portion of each of said three conductive paths of each repetitive pattern for a distance from said second edge of said base strip;

25        disposing said first reagent material on one of said three cutouts of each repetitive pattern, said second reagent material on a second of said three

cutouts of each repetitive pattern and said reference reagent material on a third of said three cutouts of each repetitive pattern, wherein said reference reagent material is made from a mixture of components in water comprising a redox mediator, said first reagent material is made from a mixture of components in water, said components being a first and second enzyme, a redox mediator capable of reacting with a product of an enzymatic reaction involving said first and second enzyme, a peroxidase capable of catalyzing a reaction involving said redox mediator and said product wherein said redox mediator is oxidized, and said second reagent material is made from a mixture of components in water, said components being said first and second enzyme, a third enzyme, said redox mediator capable of reacting with a product of an enzymatic reaction involving said first and second enzyme and said third enzyme, said peroxidase capable of catalyzing a reaction involving said redox mediator and said product wherein said redox mediator is oxidized;

drying said first reagent material, said second reagent material and said reference reagent material;

overlays a second middle layer of insulating material over and coextensive with said first middle layer, said second middle layer having a plurality of elongated cutout portions in a repetitive pattern wherein each of said elongated cutout portions exposes a corresponding repetitive pattern of said three cutouts of said first middle layer;

disposing a top layer of insulating material over and coextensive with said second middle layer, said top layer having a plurality of vent openings in a repetitive pattern wherein each of said vent openings exposes a portion of a corresponding repetitive pattern of said elongated cutout portion furthest from said first edge of said base strip; and

separating each of said repetitive pattern forming one of each of said disposable sensors.

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68. The method of Claim 67 further comprising drying said first reagent material, said second reagent material and said reference reagent material at a predetermined temperature and for a predetermined length of time sufficient to allow said first reagent material, said second reagent material and said reference reagent material to solidify and adhere to each of said electrode portion of each of said repetitive pattern of said three conductive paths.
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69. The method of Claim 68 further comprising cutting along said first edge of each of said sensors and transverse to said sensors a predetermined distance creating a sample inlet port.